REVIEW ARTICLE

Induction of Ovulation

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■ *Every effort should be made to find the cause of anovulation since specific therapy directed at correction of a specific hormonal deficiency or excess is, of course, much more effective than any empiric treatment. Moreover, some patients with disorders of ovulation may have serious, even fatal, underlying disorders. The use of thyroid or cortisone has been disappointing except in the treatment of an overt deficiency of thyroid or cortisone or an excess of adrenal androgens. Estrogens and progestational agents have not been consistently effective in the induction of ovulation. The use of clomiphene citrate, which apparently stimulates the release of gonadotropins, and the use of purified gonadotropins of human origin have been quite successful in the induction of ovulation in a variety of disorders of ovarian function. Because of real and potential hazards, the use of these new agents should be restricted to women for whom pregnancy is the primary goal or in whom standard methods of therapy have failed. Neither drug has been released by the Food and Drug Administration for routine clinical use.*

SEVERAL EFFECTIVE METHODS for the hormonal induction of ovulation in humans have been developed in the last few years. The preparation of highly purified extracts of human gonadotropins and the synthesis of pharmacologic agents which activate the release of endogenous gonadotropins are significant advances in gynecological endocrinology for which there is immediate clinical application. Use of these newer agents in the treatment of amenorrhea and other disorders of ovulation promises a much greater success rate than was thought possible a few years ago. This review will discuss these newer agents in addition to the other hormonal agents, such as desiccated thyroid, cortisone and estrogen, that are currently used for the stimulation of ovulation.

Since different mechanisms of the control of ovulation make interpretation difficult, studies in lower animals will not be discussed unless the results seem directly applicable to humans and data

from comparable studies in the human are not available. Treatment of anovulation by surgical operation, by radiation or by therapy of specific diseases associated with ovulatory failure will not be discussed except in passing. In many cases a review article or a single recent report will be cited when actually many are pertinent. The interested reader may thus be one step away from the source material. The search of the literature for this review, completed in December 1964, concentrated upon reports appearing subsequent to the publication of a series of excellent critical review articles by Kotz and Herrmann which appeared in the 1961 volume of the journal *Fertility and Sterility*.[46-51]

Indications for the Induction of Ovulation

In the patient who ovulates infrequently or not at all every attempt should be made to determine a cause and to correct any specific disorder. In many patients, however, no correctable disorder

will be found. If these patients wish to ovulate in order to become pregnant, the indication for induction of ovulation is clear and in most cases strong enough to warrant the use of methods available today. Patients with anovulation manifested by excessive or irregular uterine bleeding, amenorrhea or oligomenorrhea can now be made ovulatory and potentially fertile. This includes patients with psychogenic amenorrhea, hypothalamico-pituitary dysfunction and patients with the Stein-Leventhal syndrome of polycystic ovaries, infertility and menstrual abnormalities. Although, at present, most of these conditions are managed by therapy with estrogens or progestogens, in the future they may best be managed by repeated cyclic stimulation of ovulation even if pregnancy is not the primary goal. Since the drugs now being tested for induction of ovulation carry with them known hazards and risks as well as the unknown hazards of any new drug, use of them in unmarried patients or those not concerned with fertility is not yet warranted unless standard methods of treatment have failed. The use of ovulatory stimulants for the prophylaxis of hyperplasia or carcinoma of the endometrium or fibrocystic disease of the breast in anovulatory patients must be judged by the same standards as are now used in the evaluation of substitutional progestational therapy for prevention of these conditions.

The indications for induction of ovulation in patients who ovulate regularly are much less clear and not sufficiently strong to warrant the use of currently available methods. There is little basis for the use of ovulatory stimulants in cases of unexplained infertility except to determine beforehand the date of ovulation so insemination by the usual or by artificial means may be carried out at the appropriate time. Although the incidence of anovulatory cycles is higher in infertile patients than in the general population, this is probably accounted for by patients with irregular cycles. Since anovulation occurs in only about 5 per cent of cycles in regularly bleeding women,[46] the risks and uncertainties of present methods of ovulation induction are unwarranted in infertile patients with regular cycles and other indications of regular ovulation. Even infertile patients whose menstrual pattern is somewhat irregular or whose cycle is somewhat longer than usual probably should not now be considered for induction of ovulation if the majority of cycles are ovulatory.

TABLE 1.—*Methods for the Detection of Ovulation*

History of regular cycles	Urinary chemical assays
Basal body temperature	Physiochemical changes
Cervical mucus changes	Direct observation of
Vaginal cytology	corpus luteum
Endometrial biopsy	Pregnancy

Diagnosis of Anovulation

Since attempts at induction of ovulation should be limited to those patients with evidence of consistent absence of ovulation, brief mention will be made of methods of detection of ovulation. All infertile patients and patients with irregular menstrual cycles should be investigated for the presence of ovulation. The methods in Table 1 have been the subject of several recent critical reviews.[2,12,32,46] They are listed in the order of their clinical utility. The clinically practical methods are indirect, individually less reliable and, therefore, best used in parallel and serially.

Increased secretion of progesterone, which is produced in relatively small amounts before ovulation and corpus luteum formation, provides the basis for most of the clinically applicable methods of ovulation detection. It follows that none are useful in the prediction of ovulation. A consistent biphasic pattern of basal body temperature, with an increase of approximately 0.5 degrees Fahrenheit following ovulation caused by the thermogenic action of progesterone and its metabolites, is an excellent index of ovulation against which most other methods are judged. It should
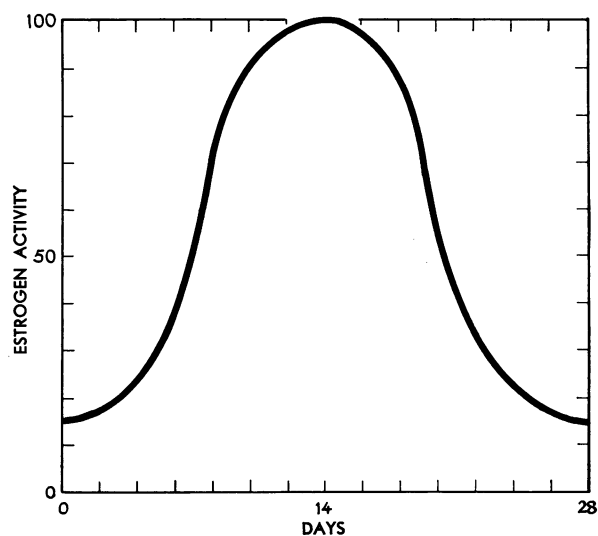

Chart 1.—Typical "haystack" pattern of measurement of estrogen activity (on a scale of 100) during menstrual cycle. The decline from ovulation on the fourteenth day is owing to antiestrogenic activity of progesterone, which decreases the biologic effects of estrogen.

be noted that the basal temperature does not indicate with any degree of accuracy the exact day of ovulation.

Increasing levels of estrogen before ovulation followed by increasing levels of progesterone after ovulation result in a "haystack pattern" when any one of a number of factors easily measured clinically is plotted against time (Chart 1). This occurs because the antiestrogenic activity of progesterone effectively decreases the biologic effects of estrogen. Increasing estrogen activity results in progressive increase in the amount of cervical mucus, its clarity, stretchability (*spinnbarkeit*) and degree of crystallization after drying (*mucus fern*). After ovulation these effects are reversed by the secretion of progesterone. The content in the mucus of glucose and of salt plays a role in the production of these characteristics and can be measured directly with simple test papers.
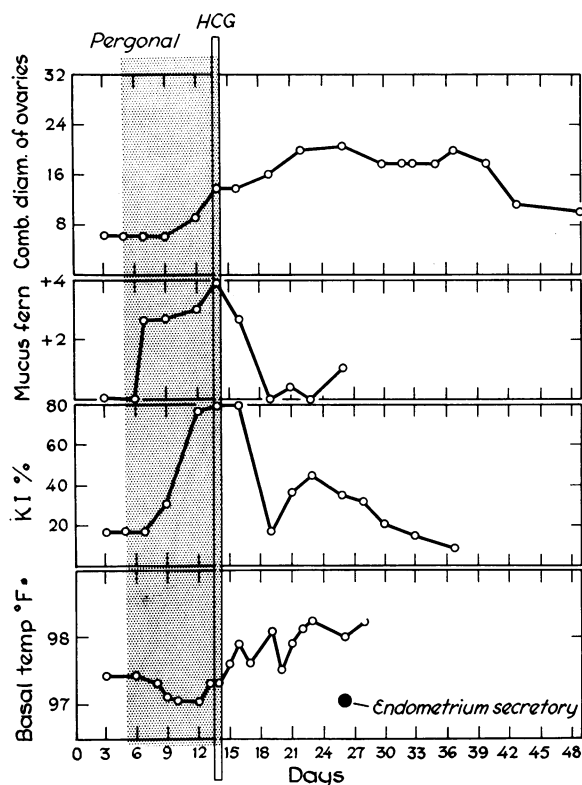
A similar haystack pattern is seen if the per

Chart 2.—Changes in clinically detectable factors in a patient treated with exogenous gonadotropins. *KI* = karyopknotic index, the per cent of desquamated vaginal cells with small dense nuclei. *Mucus fern* = degree (one plus to four plus) of fern-like crystallization of the mucus after drying. The large black dot indicates the time of endometrial biopsy. *Comb. diameter of ovaries* refers to the sum of the estimated diameters in centimeters of both ovaries; for example, bilateral 8 cm cysts = 16.

Note the persistence of ovarian enlargement during early pregnancy.

cent of mature cornified cells (cornification index, CI) or cells with pyknotic nuclei (karyopyknotic, index KI) in smears of exfoliated vaginal cells is plotted against time.

Biopsy of endometrial tissue taken late in the cycle or at the onset of menstruation will demonstrate the characteristic secretory effects of progesterone on the endometrium. The use of these clinically applicable factors in the study of an induced ovulatory cycle as shown in Chart 2.

Methods that require operative intervention for observation of a corpus luteum or recovery of an egg, and methods requiring serial determinations of pregnanediol or other steroid metabolites, although of high reliability, are obviously impractical for even one cycle. Patients studied by these methods and those who have become pregnant after isolated insemination have served to evaluate the precision of the clinically applicable methods. A variety of chemical assays of urinary metabolites and physiochemical techniques including measurement of vaginoabdominal potential differences, vulvar fluorescence, kymographic oscillations of the fallopian tubes, action potentials of the uterus, etc., have been described but have not found clinical acceptance.

Review of the standard textbooks of gynecology or endocrinology will reveal a large number of diseases which may be associated with ovulatory failure. In many, such as hypothyroidism, Cushing's syndrome, psychosis and malnutrition, ovulatory failure is a minor part of the problem and its correction will accompany successful therapy of the primary disorder. The patient will profit little from advances in endocrine therapy unless systematic efforts have been made on her behalf to be certain that there is no illness threatening her life or health. Once this has been established, nonspecific and empirical therapy may be indicated for those in whom no correctable disorder has been found. Although treatment with gonadotropins or with recently developed steroidal and non-steroidal agents may well be specific therapy for certain hypothalamic and pituitary disorders, this report will deal mainly with non-specific and empirical methods of ovulation induction.

Indirect Methods of Ovulation Induction

The following logic is the basis for a number of treatment programs designed to stimulate ovulation in anovulatory patients: severe deficiencies of

certain hormones (for example, in Addison's disease and myxedema) are associated with anovulation and amenorrhea, and replacement therapy results in resumption of ovulation; perhaps many unexplained cases of anovulation are due to subclinical deficiencies which would respond equally well to treatment with hormones, perhaps even in low doses proportionate to the severity of the deficiency.[46] Happily many cases of idiopathic anovulation are self-limited and respond to observation, placebo therapy or any one of a variety of non-specific remedies.[106] Without double-blind tests, however, interpretation of the results of therapy with these non-specific remedies is overwhelmingly difficult and, unfortunately, few such studies have been carried out. In many instances the regimens described in the literature have been proposed by enthusiasts who would feel it unfair to deprive their patients of the chance to share in the uniformly excellent results.

The proposed physiologic basis for these methods, of course, varies somewhat with the particular hormone prescribed, but in most instances is based on one of the two following premises: (1) The hormone prescribed sensitizes the ovary to respond more easily to ambient concentrations of gonadotropins, (2) the medication somehow causes the release of gonadotropins from the pituitary to cause ovulation. In some cases these effects are brought about by suppression of another hormone or factor which inhibited ovulation. In most instances there is evidence from animal experimentation to support either thesis, but, also, it must be stated that there is often equally convincing animal data to support the opposite conclusion—that is, that such treatment will inhibit ovulation. The hazard to the patient in the use of these empirical regimens is twofold: (1) adequate diagnostic studies may be omitted in the haste to provide "effective" treatment, (2) if doses beyond the physiologic range are given, the patient may then suffer from the effects of an excess of the hormone which may well include anovulation.

Thyroid

Use of thyroid extract and various synthetic or semi-synthetic analogues of thyroxin, probably the most widespread of empiric therapies for anovulation, is based on the well documented response of patients with anovulation associated with myxedema or hypothyroidism to replacement therapy with thyroid.[47,90] The drug, moreover, is a standard remedy, cheap, readily available and conveniently administered orally. A further advantage is that in reasonably physiologic dose ranges a patient with normal thyroid function will compensate for exogenous thyroid by decreasing the output of pituitary thyrotropin and thus of thyroxin from the thyroid.[28] The result is a convenient escape from many problems of overdosage. The greatest disadvantage of the empirical use of thyroid for the treatment of anovulation is that it has never been shown to be effective in euthyroid patients.[47] Suppression of thyrotropin which provides the safeguard also implies that the administration of thyroid to normal patients for the control of anovulation (or obesity or fatigue) is virtually without promise of therapeutic effect since excessive amounts would be required before elevation of the levels of circulating thyroxin or effects on metabolism would be produced.[28] Although there have been innumerable clinical reports of the use of thyroid in a variety of conditions related to ovulation, there have been few controlled studies of the use of thyroid extract in the treatment of anovulation. Even in the less well controlled studies in which the results in groups with and without treatment were compared, no effect of thyroid therapy could be demonstrated in euthyroid subjects. In cases in which there is some evidence of possible decreased thyroid function such as a low or borderline protein-bound iodine (PBI), the results are, of course, somewhat better but conclusive only in cases of true hypothyroidism.

The presence of thyroid hormone is, of course, necessary for the proper function of innumerable cellular processes, undoubtedly including those related to gonadotropin and steroid production, development of the follicle and ovulation. The effects of thyroxin on the production and release of pituitary gonadotropins and their action on the ovary has not been extensively studied. Such evidence as is available would suggest an increase in ovarian activity with low circulating thyroxin associated either with an increased secretion of gonadotropin as well as thyroid stimulating hormone (TSH) or with a diminished deactivation of gonadotropin.[61] In humans the coexistence of myxedema and precocious puberty with elevated excretion of gonadotropins has been described.[38] Production of a polycystic ovary syndrome with disorders of the estrus cycle in rats by injection of chorionic gonadotropin is possible only in animals treated with antithyroid drugs or that have

had the thyroid gland removed.[33,101] Development of the syndrome is prevented by the administration of thyroxin but, once the syndrome is established, thyroxin therapy is without effect. In several laboratory animals the administration of thyroxin will decrease the sensitivity of the ovary to exogenous gonadotropins but there is a definite variation between species.[61,68] Moreover, at low doses there seems to be some increase in ovarian response in those animals in which the response is inhibited at higher doses. Thyroxin is known to have an effect on gonadal enzyme systems which play a part in the intermediary metabolism of various androgenic and estrogenic steroids.[20] What effects these changes might have on the induction of ovulation are not clear.

Cortisone and Its Analogues

The syndrome of congenital adrenal hyperplasia caused by a deficiency of one of several enzymes necessary for the conversion of steroid precursors to cortisol is characterized by virilization and, in some varieties of syndromes, by salt loss or hypertension. A diagnostic pattern of steroid metabolites is evident in the urine at birth.[5] The syndrome of the most common type, if untreated, will result in amenorrhea in adult females. When treated with cortisone, such patients have ovulatory cycles and are fertile.

There are women who manifest one or more of the signs and symptoms of adrenal overactivity such as hirsutism, menstrual disorder, change in libido, obesity and/or elevated urinary 17-ketosteroids with normal or even relatively low excretion of 17-hydroxysteroids or 17-ketogenic steroids.* Many terms have been applied to this group of disorders but the most common is "mild adrenal hyperplasia" despite the fact that the specific enzymatic deficiencies found in congenital adrenal hyperplasia have rarely been noted in patients with virilization of adult onset except in cases of adrenal tumor.[56] Many patients with these symptoms will have disorders of ovulation, smooth, often enlarged, ovaries and will be classified as having the Stein-Leventhal syndrome.[9,27,55,60] The assumption is made that an enzymatic defect exists and that suppression of ACTH and replacement of the necessary amounts of cortisol will result in "normalization" of the altered adrenal steroid secretion and a decrease in secretion of adrenal androgens that have directly or indirectly pre-

*Reference Nos. 5, 6, 34, 36, 42, 50, 69, 91.

vented ovulation.[27,50,55,60] The finding that treatment with cortisone results in a decreased urinary excretion of 17-ketosteroids in this group of patients does not support the assumption of an enzymatic deficiency, since a similar decrease is seen in normal patients. Large numbers of patients with "mild adrenal hyperplasia" have been studied in an attempt to find a unifying theory to explain the findings. Some of them have a greater than normal sensitivity to ACTH, others have increased concentrations of potent androgens in blood and urine. Despite lack of knowledge of the exact mechanisms at work in this heterogenous group, it has been found that treatment of these patients with cortisone or its analogues will sometimes result in ovulation and fertility. If specific disorders of the gonads or the adrenal glands can be excluded by appropriate studies, a trial of cortisone therapy in the physiologic dose range equivalent to 15 to 50 mg of cortisone acetate per day is thought warranted.

The use of cortisone has been extended by some clinicians to patients with no evidence of adrenal disorder and normal urinary levels of ketosteroids.[36,39,50] Excellent results are reported but the studies are entirely uncontrolled.

The mechanism of action of corticosteroid therapy is well defined in the congenital adrenal hyperplasia syndrome: suppression of ACTH resulting in suppression of adrenal cortical production of both cortisol, and the androgenic adrenal steroids which have caused the clinical manifestations. The effectiveness of cortisone in the cases classified as "mild adrenal hyperplasia" is more difficult to explain since the physiologic basis is less well understood. The goal is the same—to reproduce the normal pattern of adrenal steroid concentrations in the blood.

To justify the use of corticosteroid therapy in patients with normal adrenal function, one must seek another mechanism of action based on the effects of excess cortisone on gonadotropic or ovarian function. Increased circulating levels of cortisone and decreased levels of the other adrenal cortical hormones and of ACTH may have effects on the gonadotropin controlling centers or directly on the ovary and its steroid synthetic mechanisms. In man, administration of cortisone results in increased urinary excretion of gonadotropins and estrogens[9] and alteration of the intermediary metabolism of estrogens and androgens.[1,107]

Estrogens and Related Compounds

Despite several encouraging reports, the use of estrogenic compounds to induce ovulation has not been popularly accepted.[48] They are, however, frequently used in the treatment of patients with anovulation since, administered cyclically in sufficient doses, they result in regular uterine bleeding accepted by the patient as a sign of normal reproductive tract function. The goal is not to stimulate ovulation but to simulate the cyclicity absent in anovulatory patients.

Oral or intravenous administration of single or intermittent high doses of estrogens has been stated to result in ovulation in a significant percentage of anovulatory patients.[52] The use of intravenous estrogen in high doses has been proposed as a test for the sclerocystic ovary syndrome since those patients who do not respond are considered to show ovulatory blockade at the ovarian level. Kupperman, the most widely quoted advocate of intravenous estrogen therapy for anovulation, reports successful induction of ovulation in over 50 per cent of selected patients. Other investigators, however, have had difficulty in duplicating these results.[48]

High dose estrogen therapy is based on the action of estrogen on the hypothalamico-pituitary control of gonadotropin secretion. A rapidly increasing concentration of estrogen—for example, that produced by the developing follicles during the normal cycle— upon reaching the hypothalamus is thought to promote the release of luteinizing hormone (LH) and suppress the release of follicle stimulating hormone. (FSH) by the pituitary. Studies of urinary excretion of estrogens and total gonadotropins in normal cyclic women show variation in which of the two reaches its peak of excretion first in the cycle.[7,43,57,99] Assays specific for LH and FSH have not yet been carried out following short term, high dose estrogen therapy in humans.

Administration of continuous low doses of estrogen has been advocated as therapy for anovulation, and excellent results have been reported in uncontrolled studies.[42,48] It has not been demonstrated, however, that such therapy is more successful than any other non-specific remedy.[25]

Estrogen therapy in continuous low doses is designed to increase the sensitivity of the ovary to gonadotropins, a response well documented in laboratory animals.[24,78,94] This sensitization may be a direct effect on the ovary or may be mediated through release of endogenous gonadotropins.[21,86,94] An analogy may also be drawn between the use of low doses of estrogen in some instances of ovarian dysfunction and low dose cortisone therapy in adrenal hyperplasia, since defects in the ovarian metabolic pathways leading to estrogen formation have been described in some anovulatory patients, especially those with hirsutism.[6,27,60]

LaBerge and Rock in 1962[53] reported their experience in the use of a stilbestrol derivative with low peripheral estrogenic activity which was thought to facilitate the release of LH. After it was administered, ovulation occurred in about one-third of more than fifty patients with ovulatory disturbances.

Clomiphene

Clomiphene (Clomid, MRL-41, MER-41 or chloramiphene) is an orally administered, non-steroidal agent synthesized by Merrell Laboratories, Cincinnati. Reports of clinical trials with the drug having first appeared about three and one half years ago, it is still under clinical investigation but will probably be considered for release by the Food and Drug Administration in the not too distant future. The structural formula is represented as follows:

STILBESTROL

CHLOROTRIANISENE (TACE)
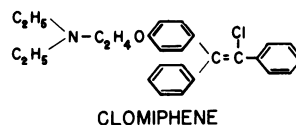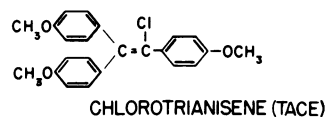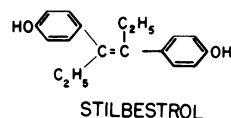
CLOMIPHENE

From its action in rats, clomiphene was expected to exhibit pituitary-suppressive and anti-fertility effects in the human. In early clinical studies it became apparent that ovulation was stimulated, not suppressed. Subsequently, induction of ovulation in women with a wide variety of disorders associated with ovulatory failure was reported.

Although non-steroidal, clomiphene has many pharmacological actions similar to those of steroidal compounds, a similarity it shares with its

chemical relatives stilbestrol and chlorotrianisene (TACE). As judged by its effects on the size of the rat uterus, clomiphene has both an estrogenic and an antiestrogenic activity.[88] Given alone, it causes an increase in uterine weight but, given along with an estrogen, such as estradiol, it inhibits the uterotrophic activity of the estrogen. This antiestrogenic activity of clomiphene is shared with other estrogens such as estrone, some progestational agents, androgens and other steroidal and non-steroidal compounds.[103a] In human females this antiestrogenic activity is manifested by regression of the estrogenic effects on the vaginal cells and cervical mucus and by the failure of exogenous estrogen to cause suppression of ovulation when clomiphene is given concurrently.[80,87]

The exact mechanism of action of the drug is a matter currently under intensive clinical and laboratory investigation. One hypothesis is that clomiphene competes with natural estrogen for the binding sites in the uterus, pituitary and hypothalamus and effectively displaces it. This mitigates the inhibitory influence of estrogen on the hypothalamico-pituitary axis and causes release of pituitary gonadotropins resulting in ovulation.[87,88]

There is considerable evidence that clomiphene does effect its ovulatory action primarily through stimulation of the pituitary gland or its hypothalamic regulators. Although it should be noted that a few investigators[11,96] have found no change in the excretion of urinary gonadotropins following clomiphene administration, most studies have demonstrated an elevation of total urinary gonadotropin excretion.[33a,73,87]

The change in gonadotropin excretion may occur several days or weeks after clomiphene administration. This has been interpreted as evidence of an indirect action through alteration of the steroid secretion pattern of the ovary. Ovarian enlargement and cyst formation, noted especially with higher doses, bear a striking resemblance to the effects seen after the administration of human gonadotropins both in gross appearance and in the effects on in-vitro steroid synthetic activity.[87,96] An increased incidence of multiple pregnancies is also common to clomiphene and gonadotropin therapy.[26,87] Patients with panhypopituitarism or with hypogonadotropic hypogonadism show diminished or absence of response to clomiphene therapy.[40,80,96] Some women experience hot flashes while taking clomiphene, especially with the larger doses.

Urinary estrogen excretion is increased in both male and female patients following clomiphene therapy.* In the few studies in which simultaneous measurements of estrogens and gonadotropins have been done no consistent relation between the patterns of excretion of the two hormones has been noted.[11,40,73,104] The same lack of correlation exists, however, in women with normal menstrual cycles.[7,43,57]

A number of findings suggest that clomiphene may cause a relative increase in the proportion of luteinizing hormone activity in addition to an increase in total gonadotropins. Increased excretion of 17-ketosteroids occurs in males but not in females, a response seen also after administration of human chorionic gonadotropins.[35,71,72,87,92] Clomiphene has been successfully used in place of human chorionic gonadotrophin (HCG) to "trigger" ovulation in amenorrheic patients stimulated with human postmenopausal gonadotropin (Pergonal).[103] There is some evidence that patients with estrogenic stimulation of the vaginal cells and endometrium, indicating follicular activity, more often respond to clomiphene therapy than those lacking follicular activity.[40] Administration of clomiphene following the rise in basal body temperature will prolong the duration of the "post-ovulatory" phase,[87] an action that can be brought about also with HCG and progestational agents. Clomiphene is not progestational in itself. Finally, studies in which urinary gonadotropin assays specific for FSH and LH have been done demonstrate a significant increase of LH activity in urine following clomiphene administration.[87]

There is also evidence for a direct action on the gonadal enzyme systems responsible for steroid synthesis in addition to its action on the pituitary-hypothalamic system. In addition to an increase in total estrogen excretion, there is an alteration in the relative proportion of the various estrogenic steroids excreted by patients following clomiphene therapy[11,96] indicating an effect on the intermediary metabolism of estrogens. Studies on placental extracts in vitro also suggest such an effect.[95]

Induction of ovulation in about 70 per cent of those anovulatory women treated has been reported (see Table 2). Best results, as would be expected, have been in patients with normal results in tests of hormonal excretion who are classified as having "idiopathic," "psychogenic" or "hypothalamic" anovulation. Patients diagnosed

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*Reference Nos. 11, 72, 73, 80, 96, 104.

TABLE 2.—*Results of Clomiphene Treatment of Anovulation as Reported by Various Investigators*

Author	Number of Patients	Number Ovulating	Number Pregnancies*
Roy[87]	179	139	31
Payne [76]	63	38†	14
Whitelaw[108]	52	41	17
Riley[80]	34	16	5
Naville[73]	36	28	8
Vorys[104]	32	26	9
Total	396	288 (73%)	84

*Note that the actual "pregnancy rate" is higher than could be calculated from this table since some of the patients were not desirous of pregnancy.
†"Improved ovarian function."

as having Stein-Leventhal syndrome have also responded in a high proportion of the cases reported. Clomiphene appears to be effective therapy for some patients with persistent lactation and amenorrhea.[40,76,87] Although success has been reported in the treatment of a few patients with elevated urinary gonadotropins,[73,76] the majority, including menopausal and climacteric patients, have not responded.[11,73,104] The dose range used in most recent studies has been 25 to 200 mg a day for one to five days. With this dosage the incidence of ovulation has been equal to that found with larger doses over longer periods of time and the side effects of ovarian cyst formation and hot flashes have been less prominent. These side effects regress spontaneously with cessation of therapy. Because of the structural similarity to MER-29 (Triparanol) which also contains the p, (β-diethylaminoethoxy)-phenyl group, potential untoward reactions regarding lens opacity or defects in cholesterol metabolism have been carefully looked for but have not been found following use of clomiphene for short term treatment.

Progesterone and Related Compounds

Progesterone and more recently the synthetic progestational steroids have been commonly used for therapy of anovulatory patients.[49] In most instances, this is designed as substitutional therapy to provoke progestational changes in the endometrium and more physiologic bleeding. The hope is often expressed that with the cyclic use of progestational agents the pituitary will be trained to a cyclic pattern or that there will be a rebound release of stored gonadotropins following cessation of therapy. This requires acceptance of the concept of inhibition of release of gonadotropin as the primary mechanism of the antiovulatory action of progestational compounds. Attempts to explain the antifertility effects of these drugs have

provided us with some information on their effects on gonadotropin production and release. In laboratory animals the inhibition of release of pituitary gonadotropins by natural and synthetic progestational agents can be demonstrated by a variety of techniques.* In humans, however, the excretion of total urinary gonadotropins is not regularly suppressed although suppression of LH seems to be more consistent.† There is also some evidence that these agents may prevent conception through a direct effect on ovarian steroid synthesis or on cervical and endometrial epithelia.‡ Recent reports of a higher incidence of multiple pregnancy occurring after discontinuation of progestational agent contraception would tend to support the theory that following cessation of progestational agent therapy there is release of an increased amount of gonadotropin.[105] There are several reports of an increased pregnancy rate after treatment of infertile patients with these drugs.[49,81,102]

In lower animals progesterone is known to facilitate ovulation under certain experimental conditions and to inhibit ovulation under others.§ Relatively small quantities of progesterone are secreted by the ovary before rupture of the follicle, but the importance of this in the mechanism of ovulation in humans is undetermined.[110] Progesterone itself given either intramuscularly or intravenously has been reported to induce ovulation in humans, but this method has never become popular.[49]

Direct Methods of Ovulation Induction

Human Chorionic Gonadotropin

Human chorionic gonadotropin (HCG) derived from the urine of pregnant women is relatively easy to obtain in large quantities, is available in standard potencies and is devoid of clinically significant toxicity. Attempts to induce ovulation with HCG have been made for many years.[10,26,51,98] Although in Europe reports of its use continue to appear, it has never been widely used in the United States for this purpose. Greater success following intravenous use has been reported recently.[52]

As was previously mentioned, if patients are first treated with other gonadotropins which manifest follicle stimulating properties, administration of HCG will result in ovulation. In intact laboratory

*Reference Nos. 4, 44, 64, 70, 77, 78.
†Reference Nos. 7, 8, 37, 57, 63, 82, 100.
‡Reference Nos. 14, 17, 22, 57, 59, 111.
§Reference Nos. 18, 21, 31, 44, 49, 64, 65, 79, 89.

rodents, HCG, acting in synergy with endogenous follicle stimulating gonadotropins, will cause ovulation and corpus luteum formation. In hypophysectomized animals, however, it will not cause ovulation but only luteinization of the theca cells of the ovary.[33,103a] Thus, in man and in experimental animals, the principal action of HCG seems to be similar to that of LH. Its success in the induction of ovulation, therefore, depends on follicular development by endogenous or exogenous gonadotropins.

HCG also has actions similar to luteotropic hormone (LTH) since administration of HCG to women following ovulation will delay the onset of menstruation, presumably by prolonging the activity of the corpus luteum. Its use in the treatment of deficient luteal phase is on this basis, although there is considerable doubt that LTH is necessary in the human during the normal cycle.

Gonadotropins of Animal Origin

Species specificity of gonadotropic hormones has delayed progress in the use of these hormones for the induction of ovulation in women. Although gonadotropins from other species are able to induce ovulation in humans, antibodies soon develop to these gonadotropins,[74] resulting in refractoriness to treatment and, in some instances, in cross reactions with human gonadotropins so that, in theory, endogenous gonadotropins are inactivated. The gonadotropin produced by the horse placenta is not readily excreted in the urine but may be obtained from the serum—pregnant mare serum gonadotropin (PMS). PMS has been used successfully for the induction of ovulation in the human—sometimes alone, but more often in combination with HCG.[51,98] Its use has been recommended as a diagnostic test of ovarian responsiveness before use of human gonadotropins.[93]

A second serious hazard to the use of PMS, perhaps related to its prolonged duration of action and poor urinary excretion, is the difficulty in establishing an effective dose. There are many reports in the European literature of massive overstimulation of the ovaries following sequential use of PMS and HCG,[112] leading to hemorrhagic corpus luteum cysts, hemoperitoneum and, in several cases, death.

Gonadotropins derived from the pituitary glands of common slaughterhouse animals have been tried in humans[41,51,54,62] and in other primates[45] with some success. Although species specificity of response is not as great for gonadotropins as for

growth hormone, it is much greater than for ACTH or insulin and the development of antibodies has been a serious problem. At present the difference in biologic activity and the risk of antibody formation prevent widespread application of even the most purified animal pituitary hormone preparation to the problem of induction of ovulation in the human.

Pituitary Gonadotropins of Human Origin

Development of methods for the isolation and purification of gonadotropins of human origin in quantities sufficient to treat substantial numbers of patients is a milestone in gynecologic endocrinology. It is a significant advance which holds promise of immediate clinical applicability for induction of ovulation in patients previously without hope of fertility. Many problems, however, are still to be met. Whatever the source of gonadotropins, the degree of purification and the resulting action in the recipient varies from batch to batch.[85] With specific bioassays as a guide, it may be possible to produce a product of consistent and predictable potency, much as the brewer or vintner blends natural products from varied sources to produce a consistent blend.

Induction of ovulation in humans by administration of gonadotropins of pituitary origin was first reported by Gemzell in 1958.[26] His preliminary work was confirmed in the United States by Buxton and co-workers in 1960.[10] Induction of ovulation was accomplished using HCG as a substitute for LH since the pituitary extracts had mainly FSH activity. Pituitary glands from 10 subjects are needed to yield sufficient gonadotropins to treat one patient for one cycle. This remains the major practical handicap to the wide employment of gonadotropins of human pituitary origin. It is of interest in this regard that the United States Public Health Service and the Veterans Administration have begun studies in which pituitary tissue obtained from a large number of hospitals is extracted and purified.[19] Members of this study group have reported methods by which several of the clinically useful pituitary proteins are extracted from fresh pituitary glands or from glands from embalmed bodies.

It is relatively easy to isolate chorionic gonadotropin from the urine of pregnant women, the concentration of gonadotropin being high during pregnancy. Pituitary gonadotropins are excreted in low concentration in urine even in castrate or menopausal subjects. Therefore, the volume of

urine required is large and the extraction and purification steps are more complex.

Three preparations of urinary gonadotropins have been obtained in sufficient quantity and of sufficient purity to be evaluated in clinical practice for the induction of ovulation.[13,15,59] One of these, the previously mentioned Pergonal, a preparation of urinary origin particularly rich in FSH activity, is under clinical investigation in the United States under the direction of Cutter Laboratories, Berkeley. The source is post-menopausal women, reportedly retired Italian nuns. The gonadotropins are extracted from the pooled urine by kaolin-acetone precipitation, concentrated and purified by several steps in which the crude precipitate is dissolved in various solvents, reprecipitated, absorbed on a Permutit® column, eluted and finally filtered through Sephadex® and freeze-dried.[16] In this state it is stable for long periods of time. Pergonal has been under clinical investigation for the past five years and has been shown to be a highly effective FSH preparation. The response to Pergonal is not decreased after repeated treatment,[83] and development of precipitating antibodies has not been demonstrated in humans and would not be anticipated. Antisera to Pergonal and other human gonadotropins, however, have been developed in laboratory animals in which they are foreign proteins.[58,66,109]

The greatest hazard of gonadotropin therapy appears to be unpredictable sensitivity or, if you will, overdosage. Enlargement of the ovaries is common and, perhaps, desirable since the pregnancy rate is higher in patients with ovarian enlargement.[26] The incidence of multiple pregnancies is greatly increased, indicating development and release of ova from many follicles. In the popular press in 1964 there have been reports of the birth of several sets of quadruplets and the premature delivery of septuplets following induction of ovulation with gonadotropins. Massive ovarian enlargement may occur from the simultaneous development of many follicles, and there have been occasional reports of ascites and hydrothorax or of rupture of the cysts requiring operative intervention.[26,29,75] Problems of dosage adjustment are currently under investigation. It has been suggested that patients be tested first with HCG alone and that those few who respond not be treated with other gonadotropins. Caution is advised also in treating patients with enlarged or cystic ovaries (Stein-Leventhal syndrome) as it is said that they are prone to development of cystic enlargement of the ovaries after gonadotropin administration. The response to human gonadotropins seems to be related to the potency of the preparation in terms of FSH, as measured by the augmentation test, rather than in terms of total gonadotropins, as measured by rat uterine or ovarian weight assays.

The preferred methods of treatment with urinary gonadotropins involve administration of the equivalent of 500 to 1,000 "units" of FSH (approximately equivalent to 120 mg of the international reference preparation, HMG 20-A) daily for eight to ten days, followed, within the next few days, by the administration of a large dose of chorionic gonadotropin such as a single injection of 10,000 to 15,000 international units or several daily injections of 3,000 to 5,000 international units each. With preparations containing little LH activity ovulation will usually not occur in the absence of HCG. Modifications of this schedule have been used in an attempt to mimic various ideas of secretory patterns of FSH and LH.[23] Following ovulation, further administration of HCG is not necessary even in women who have had hypophysectomy. The biologic half-life of HCG is such that it is excreted within a few days, implying that humans do not need luteotropic hormone activity for support of the corpus luteum after the first few days.

In Table 3 are listed representative reports of the use of human gonadotropins for the induction of ovulation. Because in several references the information was not clearly stated, the table does not include the percentages of patients or cycles in which attempts to stimulate ovulation were successful. Comparisons with other methods of treatment should not be made since many of the patients treated with gonadotropins are those with long-term amenorrhea which was not benefited by treatment with other agents. Several of the pa-

TABLE 3.—*Results of Treatment of Anovulation with Human Gonadotropins as Reported by Various Investigators*

Author	Source of Gonadotropins	Patients	Number Pregnant
Gemzell[26]	Pituitary	35	18
Buxton[10]	Pituitary	11	3
Meares[67]	Pituitary	3	0
Bettendorf[3]	Pituitary	19	0
Crooke[13]	Pituitary and urinary	9	6
Diczfalusy[15]	Pituitary and urinary	6	2
Rosemberg[84]	Urinary	11	1
Paseto[75]	Urinary	14	2

tients successfully treated had severe pituitary deficiency. In addition several of the reports deal with trials of varying dosage schedules of new preparations. Once a suitable schedule was established, ovulation could be induced in the majority of patients.

The Future

What lies ahead in the field of ovulation induction? Several possibilities are suggested by recent work in laboratory animals. At present they are purely speculative.

A gonadotropin-releasing factor of relatively simple chemical structure but specific physiologic activity has been obtained from the hypothalamus. Purification or synthesis of such a compound might provide another direct means of ovulation induction in the human by regulation of the release of LH.[64]

The amino acid sequences of ACTH and related pituitary protein hormones have been identified and several physiologically active polypeptides, representing parts of the molecule, have been synthesized. The methods used in this achievement are being directed to the study of the gonadotropins and other pituitary hormones with a goal of eventual synthesis of the hormones to provide a practical source sufficient to meet the demand. Perhaps minor alterations of the protein hormones derived from animal pituitary glands could be accomplished with maintenance of physiological activity but loss of antigenicity.

A gonadotropin inhibiting substance has been identified in urine and found to vary in concentration under various conditions.[97] The role this factor plays in the physiology of the reproductive cycle and the pathophysiology of some types of anovulation is still under investigation. It does not seem overly presumptive to expect the development of competitive antagonists or antibodies to this substance which might be of use in the induction of ovulation.

Research in the neurophysiologic control of LH release has yielded information about specific centers in the hypothalamus which, upon electrical stimulation, produce the gonadotropin releasing factor mentioned above and result in the release of LH into the circulation.[21,64,89] It has been suggested that, even in animals other than those (such as the rabbit) which ovulate only after copulation, there may be reflexes involving these centers which can be initiated by external stimulation and

result in the release of gonadotropins. How far into the world of science fiction do we go to envision the induction of ovulation by manipulation or electrical stimulation?

Development of a safe, cheap, consistently effective, oral preparation for the induction of ovulation by whatever mechanism would be of considerable interest and would allow the use of this therapy for conditions in which fertility is not a consideration and in which substitutional therapy with progestational agents is now employed. Moreover, such an agent would be of considerable interest to the estimated five million young Catholic couples in the United States. Many of them find the use of the rhythm system of contraception ineffective or impractical because of irregular cycles and difficulty in accurately determining the time of ovulation.[30] Since the Roman Catholic Church has not, as yet, authoritatively approved for use by its members the ovulation-suppression or mechanical methods of contraception, many women who do utilize one of these methods are disturbed by the side effect of reduced tranquility which accompanies the reduced fertility. It seems reasonable, at this moment, that a method utilizing ovulation induction for improving the effectiveness of the rhythm system would be acceptable to at least a large segment of Catholic theologians.

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